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Binding properties of a streptavidin layer formed on a biotinylated Langmuir–Schaefer film of unfolded protein

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ABSTRACT

A Langmuir monolayer of carbonic anhydrase (CA) unfolded at an air/water interface was transferred onto the hydrophobic surface of a silicon wafer by means of the Langmuir–Schaefer technique. The transferred CA film was biotinylated and was incubated in a streptavidin (SAv) solution to obtain a densely packed SAv layer by biotin–SAv linkage. Biotinylated proteins including ferritin, catalase, alcohol dehydrogenase, and carbonic anhydrase were incubated with the SAv layer and binding of these proteins was examined by atomic force microscopy. High-density binding of the biotinylated proteins was observed, whereas the amount of adsorbed non-biotinylated proteins was low or negligible. The SAv layer on the Langmuir–Schaefer film of unfolded protein could become a basic architecture for protein immobilization studies.

1. Introduction

High-density arrays of proteins immobilized on a solid surface would be useful for both basic and applied studies. Various immobilization techniques have been developed [1–3]. Physical adsorption of proteins directly onto glass or silicon surfaces might be the simplest process, but it has disadvantages such as denaturation, random orientation, and poor stability under various buffer conditions [4]. Covalent immobilization utilizes the binding between amino acid side chains and a solid surface activated with reactive groups such as N-hydroxysuccinimide (NHS) esters and amines [5,6]. Oriented immobilization is desirable if the protein chip requires high sensitivity for detection of target molecules. Reactive groups genetically introduced at specific positions of the protein surface allow covalent reactions with reactive groups introduced on the solid surface [7]. Affinity immobilization is another method for oriented immobilization. A poly-His tag genetically introduced at the C- or N-terminus of the protein allows binding with a nickel nitrilotriacetate functionalized surface [8,9]. Biotin–streptavidin (SAv) linkage is also exploited for protein immobilization. SAv consists of four subunits, each of which has an extremely high affinity for biotin with a dissociation constant (K_d) of ~10^{-15} mol/L [10]. Biotinylated proteins are presumably bound to a layer of SAv. Affinity binding of SAv to a biotinylated thiol monolayer on a gold surface was used to obtain high density adsorption of SAv molecules [11,12].

We have shown that a two-dimensional crystal of SAv can be obtained by adsorption to a film of synthetic polypeptide (poly-benzyl-histidine, PBLH) spread at an air/water interface [13]. The PBLH/SAv film can be transferred onto a hydrophobic silicon wafer or glass cover slip [14], and biotinylated proteins are bound to this SAv crystal surface [13]. In our recent work, proteins were directly spread at the air/water interface. When the spread protein concentration was high, the protein Langmuir film had a mosaic structure in which both globular and unfolded protein forms coexisted, and the interfacial properties were dependent on the proteins themselves [15]. In a time-course study of carbonic anhydrase (CA) Langmuir films by atomic force microscopy (AFM) imaging, the surface density of the globular form of CA decreased with time, indicating unfolding of the intact CA that occurred on a time scale of minutes. In the present study, the Langmuir film comprising unfolded CA was transferred onto a silicon wafer by using the Langmuir–Schaefer technique [16,17]. The Langmuir–Schaefer film of CA (LSF-CA) was biotinylated and then utilized for preparing a dense packing of SAv via biotin–SAv linkage. Proteins that were randomly biotinylated at their surface were bound to the densely packed SAv layer. AFM imaging of these samples revealed two-dimensional (2D) molecular packing of biotinylated proteins at molecular resolution. Thus, the present study reports an easy method to prepare an SAv layer that has a high affinity for biotinylated proteins with simultaneous weak nonspecific adsorption of non-biotinylated proteins.

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2. Materials and methods

2.1. Materials

All of the proteins used in the present study including alcohol dehydrogenase from *Saccharomyces cerevisiae* (AD, 141–152 kDa, A3263), carbonic anhydrase from bovine erythrocytes (CA, 29 kDa, C3934), catalase from bovine liver (CAT, 250 kDa, C3155), ferritin from equine spleen (Ferr, 480 kDa, F4503), lysozyme from chicken egg white (Lyz, 14 kDa, L6876), and streptavidin from *Streptomyces avidinii* (SAv, 60 kDa, S4762) were purchased from Sigma-Aldrich, Japan. The lyophilized proteins were dissolved in pure water produced by using a Direct-Q UV3 system (Merck Millipore, Japan) and passed through a membrane filter (Ultrafree-MC, 0.1-μm pore size, Merck Millipore, Japan) before use. Thymol contained in the catalase solution was removed by washing using a centrifugal filter (Amicon Ultra 0.5 mL, Merck Millipore, Japan) before biotinylation.

An Si wafer [n-type (111) with resistivity of 10 Ω cm, purchased 25 years ago from Sumitomo Sitix, Japan] was cut into pieces with dimensions of 4 × 5 ~ 5 × 6 mm and rendered hydrophobic by treatment with the silylation reagent hexamethyldisilazane (Nacalai Tesque, Japan) in the gas phase at 60 °C for 30 min after sonic cleaning in neutral detergent followed by deep UV (185 nm) cleaning.

2.2. Biotinylation of proteins

Lysine residues on the surface of test proteins were biotinylated using sulfo-NHS-LC-biotin (BioVision, USA). Biotinylation was performed at room temperature in 100 mM phosphate-buffered saline (PBS, pH 7.4, 150 mM NaCl) with a protein concentration of 2–10 mg/mL and sulfo-NHS-LC-biotin at 1 mg/mL. The reaction mixture was incubated at room temperature for 1 h and the reaction was terminated by adding 100 mM Tris (pH 8.2). Non-reacted and hydrolyzed biotin reagent was removed by ultracentrifugation using Amicon Ultra 0.5 mL centrifugal filters with at least six cycles of concentration and dilution with PBS, with each centrifugation for 10 min at 14,000 ×*g*, with a concentration factor of ~20.

2.3. Binding of proteins to the SAv layer formed on the biotinylated LSF-CA

The novelty in the present study is the use of a biotinylated Langmuir–Schaefer film of protein CA (LSF-CA) for preparing a densely packed array of SAv by biotin–streptavidin linkage (Fig. 1).

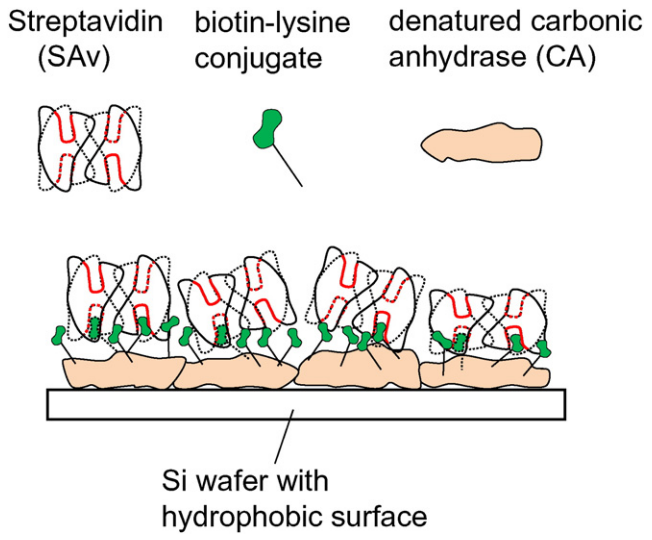

Fig. 1. SAv layer formation via linkage to the biotin conjugated with the surface lysine of a Langmuir–Schaefer film of carbonic anhydrase (LSF-CA).

Three micro-troughs with dimensions of 2.0 × 1.7 × 0.2 cm were milled on a thin plate of Delrin plastic (Fig. 2A). A CA solution with a concentration of 0.5 mg/mL was spread over the subphase of 20 mM phosphate buffer (pH 5.4) using a 10-μL microsyringe (#701 Hamilton) to create a monolayer. Continuous flow of the CA solution was attempted by keeping the tip of the microsyringe needle in contact with the subphase surface for 10–20 s. Spreading of 1 μL of the CA solution corresponded to a surface density of the CA monolayer of 1.5 mg/m^2. The surface film of CA was incubated at the air/water interface for 20 min after spreading. The CA Langmuir film was transferred onto the Si wafer by bringing the wafer horizontally into contact with the surface film (Langmuir–Schaefer method, Fig. 2B) for 5–10 min. Usually, two chips of the wafer were deposited on each trough, and therefore, up to six samples of LSF-CA were prepared simultaneously. The wafer chips were recovered from the subphase surface and rinsed with pure water. The LSF-CA was biotinylated in a PBS solution of sulfo-NHS-biotin (1.0 mg/mL, BioVision) for 30–60 s (approximated as 1 min in the following) or a longer period of 20 min. The LSF-CA was then rinsed twice with 100 mM Tris pH 8.4 and 100 mM PBS pH 7.4 and then with pure water. To prepare the SAv layer on the biotinylated LSF-CA, the wafer chip was incubated in a solution of SAv with a concentration of 0.1 mg/mL for 3 min. All of the preparation processes for the proteins deposited on the Si surface and on the Langmuir trough were performed at ~24 °C.

The SAv layer obtained was preserved in glucose as follows. After recovery from the SAv solution and rinsing with pure water, the wafer chip was rinsed with a 3% glucose solution. The wafer chip wetted with the glucose solution was transferred into a 1.5-mL Eppendorf

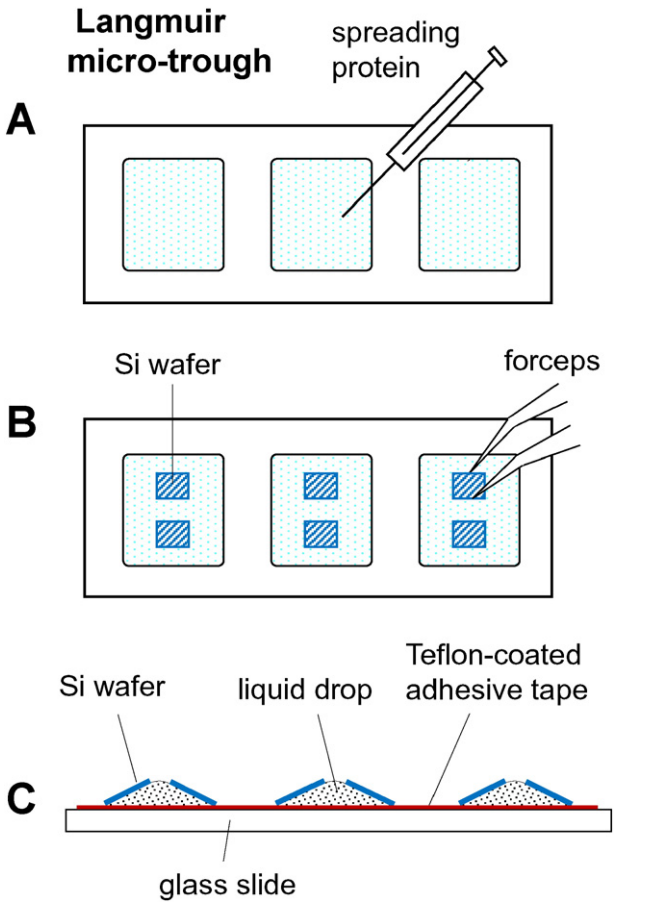

Fig. 2. Preparation of SAv layers on a Si wafer. (A) Spreading Langmuir monolayer of CA, (B) transfer of the CA Langmuir film onto the Si wafer by horizontal deposition (Langmuir–Schaefer method), (C) rinsing, biotinylation, and SAv binding using liquid drops (150–180 μL) maintained on Teflon-coated tape.